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Formation of *Pisolithus* Ectomycorrhizae on Loblolly Pine Seedlings With Spore Pellet Inoculum Applied at Different Times

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Formation of *Pisolithus* Ectomycorrhizae on Loblolly Pine Seedlings With Spore Pellet Inoculum Applied at Different Times

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ABSTRACT

Spore pellets of Pisolithus tinctorius (Pt) were applied at 4.05 or 8.10 g/m² to fumigated soil in loblolly pine microplots at April sowing and monthly through September 1983. Vegetative inoculum of Pt applied before sowing was used for comparison.

Pt indices of 82 to 90 for seedlings with vegetative inoculum were significantly greater than indices for seedlings with Pt pellets. Pellet applications at the high rate increased Pt indices by 14, 43, and 68 percent when applied at sowing or in May and June, respectively. High and low rates of application at sowing and the high rate at May were the only pellet treatments to result in Pt indices of >50 for seedlings by lifting time in January. Effectiveness of spore pellets applied after May decreased as naturally occurring ectomycorrhizae increased on seedling roots. Competition for short roots after May between Pt spore and native spore inocula resulted in Pt indices of <50.

Keywords: Seedling quality, Thelephora terrestris.

Various types of Pisolithus tinctorius (Pers.) Coker & Couch (Pt) inocula have been developed in recent years to form specific ectomycorrhizae on container-grown and bare-root pine seedlings in nurseries. Vegetative (mycelial) inoculum has proved to be effective in forming Pt ectomycorrhizae on tree seedlings in a variety of nurseries, and industrial fermentation procedures have been developed to produce the inoculum (Marx and others 1982, 1984a). A commercial nursery bed seeder has also been modified to inoculate nursery seedbeds with different formulations of vegetative inoculum (Cordell and others 1981).

Basidiospores of Pt have also been shown to be effective inoculum in nurseries. Fruit bodies of Pt containing basidiospores occur naturally in great abundance on pines and oaks growing on disturbed and adverse sites in the United States and abroad. Spore inoculum has been used with a variety of procedures to form ectomycorrhizae on various tree species. To inoculate bare-root seedlings, spores can be mixed with hydromulch, added directly into soil, dusted onto soil or seedlings (Alvarez and Trappe 1983; Marx 1976; Marx and Bryan 1975; Marx and others 1979), or mixed with the pelletizing matrix of encapsulated seed of different pine species (Marx and others 1984b).

Inoculating with Pt spores is a problem because they are dry, readily airborne, and difficult to handle during inoculation. Recently, we found that Pt spores could be bound with special adhesives to the exerior of small vermiculite particles (2 to 4 mm dia.); spores remain fixed during handling. Preliminary tests indicated that Pt spore pellets were effective inocula for ectomycorrhizal development on bare-root and container-grown pine seedlings (unpublished data). A potentially important use of Pt spore pellets is during the growing season, not only at sowing time in the nursery. This option could prove valuable to the user if seedlings inoculated during the growing season form sufficient amounts of Pt ectomycorrhizae by seedling dormancy. For example, a nurseryman could accept and fill orders for Pt "tailored" seedlings perhaps as late as 2 to 3 months after sowing, whereas present technology with vegetative inocula dictates that the nurseryman must have

orders prior to sowing in the spring when inoculations are normally done. Many users of tailored seedlings may not know their needs until later in the season. However, the measure of success of midseason inoculation, as with any inoculation, is the amount of Pt ectomycorrhizae present on roots at lifting time. Research has shown that southern pine seedlings planted on reforestation sites must have half or more (Pt index >50) of their ectomycorrhizae formed by Pt if they are to exhibit significant increases in survival and growth over routine nursery-grown seedlings with naturally occurring ectomycorrhizae (Kais and others 1981; Marx and others 1981; Ruehle and others 1981).

The purpose of this study was to determine if Pt spore pellets applied at different rates at different times during the growing season are able to form suitable amounts of Pt ectomycorrhizae on loblolly pine (Pinus taeda L.) seedlings grown in nursery microplots containing fumigated soil.

Materials and Methods

Seventy wood-frame microplots (0.6 x 0.6 x 0.6 m) were placed 1.5 m apart on level ground in five blocks of 14 microplots each. Each microplot was filled to a depth of 20 cm with gravel and fumigated with methyl bromide. A soil mixture of forest clay loam, sand, and milled pine bark (2:1:1, v:v:v) was fumigated on a concrete pad and then used to fill the microplots. All fumigation was done with Dowfume MC-2 (Dow Chemical Co., Midland, MI) at 1 kg/18 m^2 of soil surface (25-cm deep) under clear polyethylene plastic for 48 hours. During fumigation, soil moisture was approximately 50 percent of field capacity and soil temperatures ranged from 26 to 38 °C. After fumigation, the soil mixture contained 8 µg/g of available P, and 84, 110, 20, and 18 µg/g of exchangeable K, Ca, Mg, and Mn, respectively. Total N was 156 µg/g, organic matter was 2.1 percent, and pH was 5.1.[1]

Vegetative inoculum of Pt (isolate 288) was prepared as previously described (Marx and Rowan 1981). Final bulk density of the dried inoculum was 314 g/liter with 45 percent moisture; pH was 4.3. Dried inoculum contained 0.9 mg glucose/g.[2] Inoculum was stored at 5 °C for 5 days before use. This inoculum (IMRD) was used to compare with Pt spore pellet treatments.

Spore pellets were prepared with spores of Pt collected in late summer 1979 from fruit bodies found on acid coal spoils in central Alabama. There were 125 to 135 pellets/g and each pellet contained approximately 3 x 10^6 spores (2.5 to 3 mg). Two rates of application were used. The low rate was 1.5 g pellets/microplot (0.36 m^2), which is equivalent to 4.05 g/m^2. The high rate was twice the low rate.

IMRD inoculum at a rate of 0.504 liter/m^2 of soil surface and granulated commercial 10-10-10 fertilizer at 560 kg/ha were broadcast evenly over the soil in one microplot/block and manually mixed into the upper 10 to 12 cm of soil. The remaining 13 microplots/block received only fertilizer. Loblolly pine seed (Livingston Parish seed source) were stratified for 30 days, air dried, and treated with Arasan® + latex sticker. One hundred and twenty seed were evenly distributed in four rows 0.6 m long, spaced 12 cm apart in each microplot, and covered with 5 mm of soil. Fumigated pine straw was placed 2 cm deep over the soil as a mulch. Immediately after sowing and mulching (April 18, 1983), two microplots/block were randomly selected and inoculated at either the low or high rate of Pt spore pellets. Spore pellets were broadcast evenly over the mulch and within 2 hours all microplots were irrigated with 10 mm of water. This inoculation procedure was repeated in mid-May, June, July, August, and September. One microplot/block remained free of artificial inoculation to serve as a control. The experimental design had 14 treatments: 2 rates of Pt spore pellets x 6 inoculation periods, IMRD inoculum, and a control, replicated 5 times in a randomized block design.

Prior to inoculation in May through September, five randomly selected seedlings/microplot were carefully removed,

[1,2]Analyses were done by A&L Agricultural Laboratories, Inc., 411 North Third Street, Memphis, TN.

washed, and their roots visually assessed quantitatively and qualitatively for ectomycorrhizae formed by Pt and naturally occurring fungi (Marx and Bryan 1975).

During the growing season, seedlings were irrigated twice weekly (2.5 cm/wk) and broadcast fertilized with 56 kg of N/ha (as NH_4NO_3) in early June and again in early August. Seedling foliage was sprayed weekly from mid-June through mid-September with dimethoate at the recommended rate to control insects. Microplots were examined twice weekly beginning in late July, and the number of fruit bodies of *P. tinctorius* was recorded. Fruit bodies were removed from the microplots to reduce spore contamination of other microplots.

After seedling dormancy in January 1984, roots were vertically cut between rows and undercut 20 cm deep with a shovel. Seedlings were removed by hand, and their roots were washed in water. All seedlings were examined for specific ectomycorrhizae and then rated for morphological grade. Seedlings with less than 12 cm height, 2 mm root-collar diameter, and without secondary needles were rated culls and were discarded; the remaining seedlings were considered plantable. Ten plantable seedlings/microplot were selected at random and measured for height, root-collar diameter, and top and root fresh weights; root systems were visually assessed for specific ectomycorrhizae. Data on ectomycorrhizae were integrated into a Pt index by the formula $a \times (b/c)$ where a = percent of assessed seedlings with Pt ectomycorrhizae, b = average percent of feeder roots with Pt ectomycorrhizae (including 0 percent for seedlings without Pt), and c = average percent of feeder roots with ectomycorrhizae formed by Pt and other fungi (total ectomycorrhizal development). Data were evaluated by analysis of variance, and significant means were separated with Duncan's multiple range test (P = 0.05).

Results and Discussion

Because of soil disturbance in each microplot caused by the monthly removal of five seedlings, growth of remaining seedlings could not be considered representative of treatment. At lifting, seedling measurements averaged 27 cm in height, 5.8 mm in root-collar diameter, 15.5 g in top fresh weight, and 5.3 g in root fresh weight. However, seedlings with Pt ectomycorrhizae formed by IMRD inoculum were significantly larger in all growth measurements than seedlings in other treatments. There were no significant differences in percentage (6.9 to 12.3) of cull seedlings due to treatment.

Monthly assessments of control seedlings showed that naturally occurring fungi progressively formed significantly more ectomycorrhizae each month until September, then the development stabilized at about 60 percent of short roots colonized (fig. 1). The Pt index of seedlings in the IMRD inoculum treatment was 21 as early as June, then doubled to 42 by July, and more than doubled again to 90 by August. There was no significant change in Pt index after the August assessment.

Seedlings treated at the low rate of Pt spore pellets applied at sowing had the same Pt indices in June and July as those with IMRD inoculum but did not exceed 65 for the remainder of the year; these latter indices were significantly lower than those from IMRD inoculum. Pellet inoculations in May at the low rate resulted in seedlings with a Pt index of 42 by September, after which it remained unchanged. Seedlings inoculated in June or later at the low rate of pellets did not have Pt indices >24 at any assessment time.

Seedlings treated with Pt spore pellets applied at the high rate at sowing had the same Pt index in June as those with IMRD inoculum. By July the Pt index for these seedlings reached 65, which was significantly greater than that for seedlings with IMRD inoculum. However, by August this index stablized at 72, a value significantly lower than that for seedlings with IMRD inoculum. This index did not change for the balance of the year. Inoculations of seedlings in May at the high rate resulted in a Pt index of 52 by August and an index of 67 by September, a value which became no higher thereafter. Inoculations of seedlings in June resulted in a

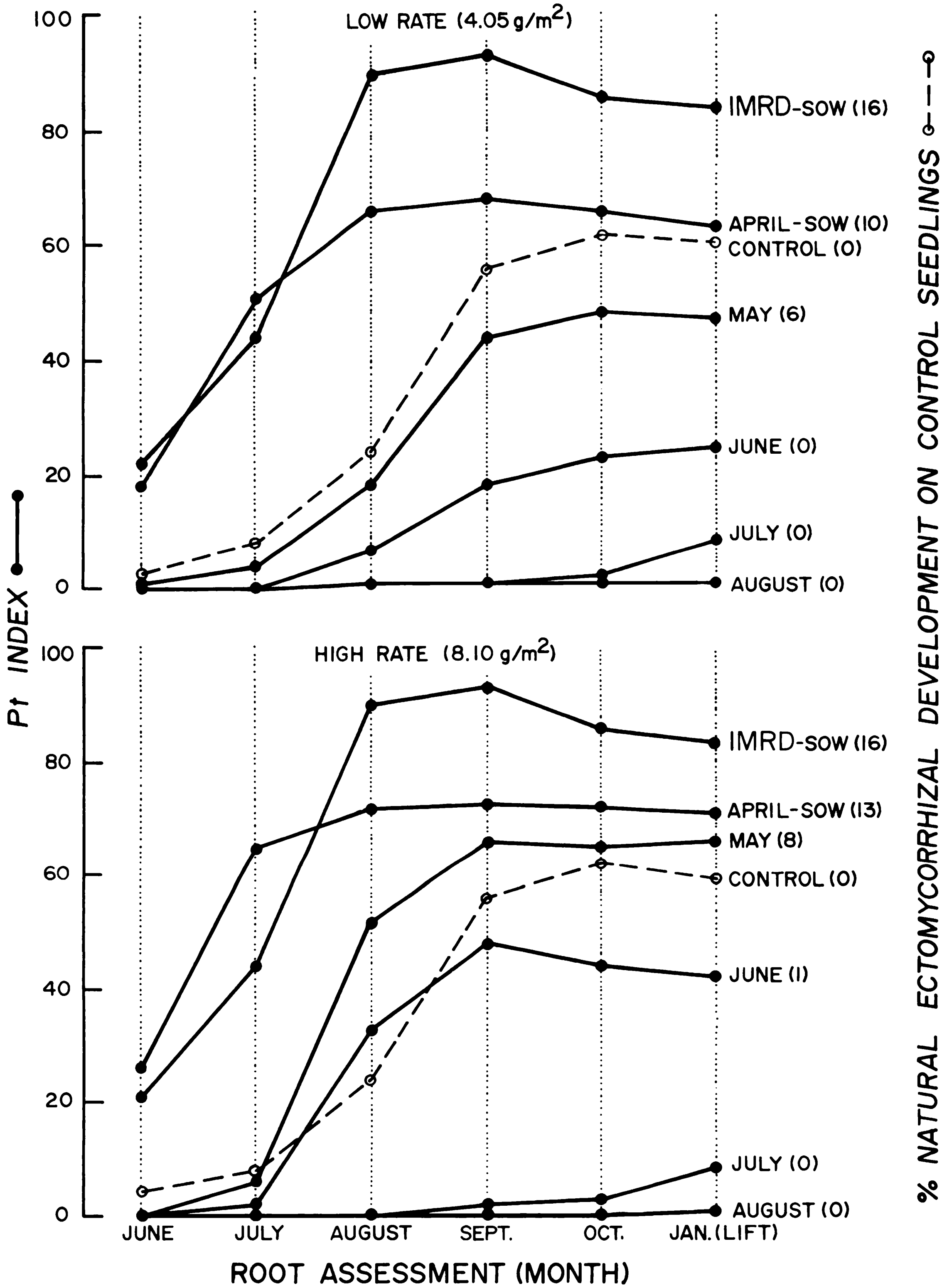

Figure 1.--Development (Pt index) of Pisolithus tinctorius and naturally occurring ectomycorrhizae on loblolly pine seedlings after application of Pt spore pellets at two rates applied at monthly intervals. "Sow" indicates inoculation was done at time of sowing seed. Numbers of fruit bodies of Pt produced through the growing season in all microplots of the specific treatments are in parentheses.

Pt index of 48 by September, after which it did not change. Seedling inoculations in July or later at the high rate resulted in Pt indices >9 at any assessment time.

Spore inocula did not result in Pt indices as high as those from mycelial inoculum later in the growing season even though their earlier Pt indices were the same or greater than mycelial inoculum. There are two possible explanations. First, spores may be leached by irrigation water or rain from the root zone with time. New Pt ectomycorrhizal formations would result primarily from root-to-root spread of earlier formations. Mycelial inoculum contained in relatively large (3 to 8 mm) vermiculite particles is not leached from the root zone, and new ectomycorrhizal formations can arise from roots penetrating this inoculum as well as from root-to-root spread, which result in higher Pt indices. Second, spores collected from many different fruit bodies are highly diverse genetically. Mycelial inoculum is derived from a single strain which has been improved through the years by repeated host passage (Marx 1981). The mycelial strain may be genetically and, thus, physiologically superior to wild-type spores for artificial inoculations in tests of this type.

Total fruit body production by Pt was related to Pt index, inoculum type (vegetative or spore), and time of inoculation. Inoculum treatments resulting in the highest Pt indices for seedlings also produced the greatest number of fruit bodies. Those treatments resulting in Pt indices >40 by July produced more than 70 percent of all fruit bodies.

These results show that as natural ectomycorrhizal development increases over time, the effectiveness of Pt spores in pellet form decreases with later inoculations. This is apparently due to two factors: (1) fewer nonmycorrhizal short roots available for colonization by Pt spores, and (2) less growing season remaining for spores to be effective in forming ectomycorrhizae. The latter factor may be of lesser significance than the first since inoculation with pellets at either rate in April or May resulted in much higher Pt indices 2 months after inoculations than did later inoculations assessed 4 to 5 months afterward. The results suggest that soil and environmental conditions (temperature, increasing daylength, etc.) and seedling susceptibility are more conducive to ectomycorrhizal development in the early part of the growing season.

Ruehle (1980) reported similar competition between spore inoculum of Pt and naturally occurring ectomycorrhizal fungi on container-grown loblolly pine seedlings. He reported that seedlings inoculated at 0, 2, 4, 6, and 8 weeks of age with Pt spores had progressively fewer Pt ectomycorrhizae by more naturally occurring ectomycorrhizae by week 18. He concluded that early natural ectomycorrhizal development precluded significant development of Pt ectomycorrhizae when Pt spores were applied later than at sowing.

Doubling the amount of Pt pellets resulted in a 14, 43, and 68 percent increase in Pt indices for seedlings when pellets were applied at sowing, in May, or in June, respectively. However, inoculation at sowing at the low and high rates and inoculation in May at the high rate were the only pellet treatments resulting in a Pt index >50 at lifting time. A Pt index >50 is the minimum standard of inoculum effectiveness because only groups of seedlings with this or greater indices have shown measurable improvements in field performance over routine nursery seedlings on reforestation sites in the South.

The results indicate that Pt spore pellets have the potential to be used as effective inoculum resulting in Pt indices >50 for loblolly pine seedlings in fumigated nursery soil. Pellets must be applied at sowing or not later than 1 month after sowing so that Pt can effectively colonize roots before significant competition for short roots by naturally occurring ectomycorrhizal fungi occurs. Results of previous research on ectomycorrhizal fungus inoculation (Marx and

others 1984a) in microplots used in the present research indicate that results in microplots are consistently better than those in conventional nurseries. Research on effectiveness of Pt spore pellets should be done in these nurseries before widespread use of spore pellets is considered as a viable alternative to other inoculum formulations.

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Pt spore pellets applied at 4.05 g/m^2 or 8.10 g/m^2 at sowing and the highest rate in May produced effective Pt indices (Pt index >50) by lifting time. Other dates of application were ineffective (Pt index <50) due to competition for short roots from native spore inocula.

KEYWORDS: Seedling quality, *Thelephora terrestris*.

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